Research Highlight #137

A Model for the Solution Structure of Visual Arrestin

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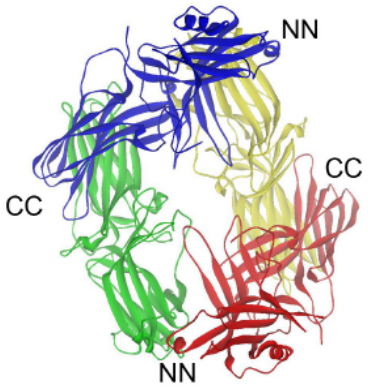
G-protein coupled receptors (GPCRs) are the largest known family of signaling proteins and the target of more than 50% of the current pharmaceuticals on the market today. Arrestins are a small family of proteins that play a key role in the regulation of GPCRs. Specifically, visual arrestin is unique in that it is responsible solely for the inactivation of rhodopsin. Visual arrestin concentration within rods can reach 1 mM, and it has been shown that it cooperatively forms tetramers in solution. Plus, two crystal structures show that arrestin crystallizes as a tetramer. The monomer-dimer and dimer-tetramer equilibria are in the experimental range of EPR spectroscopy, making it possible to selectively study monomers and tetramers in solution.

In order to establish the biological function of visual arrestin, it is important to identify its structure as a monomer, dimer, and tetramer in solution.

The aim of this ongoing collaborative research is to characterize the solution structure of visual arrestin, as several aspects of its structure in solution remain unknown, and to examine its interactions with rhodopsin. Our experimental data using site-directed spin labeling (SDSL) and both CW and DEER spectroscopies do not align with the tetramer found in the published crystal structures (*e.g.*, Ref. 1). Therefore, in the studies highlighted here, we explored the structural topology of the arrestin tetramer in solution using a combination of experimental EPR and light-scattering data and computer modeling. Then, in order to test the models generated, additional experimental testing was done by observing spin-label mobility differences at proposed interface and noninterface sites under monomeric and tetrameric concentrations and by disulfide cross-linking. DEER was also utilized for observing distances across the tetramer between spin-labeled sites and to identify the lack of oligomerization in truncated versions of the protein.



The model that met all of the experimental criteria is shown on the right [2]. Our model forms a closed, circular, and symmetric tetramer, which aligns with the experimental data showing that higher order oligomers are not formed, and is unlike the crystal tetramer, which appears as if it is able to form higher order oligomers. It also fits with previous data showing that only the monomeric form of arrestin binds to rhodopsin, as all of the rhodopsin-binding sites (identified by SDSL as being on the concave surfaces of arrestin) [3] are unavailable in the tetramer model we identified. This is a significant contribution to the study of arrestin structure and function that takes advantage of a powerful interactive combination of EPR capabilities, biological data, and advances in computer modeling.

[1] S.M. Hanson, N. Van Eps, D.J. Francis, C. Altenbach, S.A. Vishnivetskiy, C.S. Klug, W.L. Hubbell, V.V. Gurevich. Structure and function of the visual arrestin oligomer. EMBO J. 26 (2007) 1726-1736.

[2] S.M. Hanson, E.S. Dawson, D.J. Francis, N. Van Eps, C.S. Klug, W.L. Hubbell, J. Meiler, V.V. Gurevich. A model for the solution structure of the rod arrestin tetramer. Structure 16 (2008) 924-934.

[3] S.M. Hanson, D.J. Francis, S.A. Vishnivetskiy, E.A. Kolobova, W.L. Hubbell, C.S. Klug, V.V. Gurevich. Differential interaction of spin labeled arrestin with inactive and active phophorhodopsin. Proc. Natl. Acad. Sci. USA 103 (2006) 4900-4905.